PB85-249852

Glutathione Plus Cytosol- and Microsome-Mediated Binding of 1,2-Dichloroethane to Polynucleotides

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Cincinnati, OH

1985

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TECHNICAL REPORT DATA
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1. REPORT NO. EPA/600/J-85/139	2.
3 PB85-249852	
4. TITLE AND SUBTITLE GLUTATHIONE CYTOSOL- AND MICROSOME-MEDIATED BINDING OF 1,2-DICHLOROETHANE TO POLY-NUCLEOTIDES (Journal Version)	5. REPORT DATE 1985
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Edith L. C. Lin, Joan K. Mattox and Michael A. Pereira	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency 26 W. St. Clair Street, Cincinnati, OH 45268	10. PROGRAM ELEMENT NO. ABMC1A
	11. CONTRACT/GRANT NO. In-House
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, North Carolina 27711	13. TYPE OF REPORT AND PERIOD COVERED Journal Article
	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES
Journal: Toxicology and Applied Pharmacology, 78, 428-435, (May 1985).
Project Officer: Edith L. C. Lin, Ph.D., TMD, HERL (CI)

16. ABSTRACT 1,2-Dichloroethane-[1,2^{14}C] was metabolized by rat liver microsomes to products that irreversibly bound polynucleotides. The polynucleotides were then enzymatically hydrolyzed and the products separated by HPLC equipped with an ODS or a SCX column. The products of microsome mediated binding were identified in the HPLC eluate as 1,N^6-ethenoadenosine to polyadenylic acid, 3,N^4-ethenocytidine to polycytidylic acid, and two cylic derivatives to polyguanylic acid. 1,2-Dichloroethane-^{14}C was also metabolized in the presence of a glutathione-cytosolic fraction and a polynucleotide. After enzymatic hydrolysis of the polynucleotide, the major peak of radioactivity was eluted from a Sephadex G-25 column in the salt volume which would exclude the presence of a product containing both GSH and a nucleoside. Chromatography by ODS-HPLC of the major peak from Sephadex G-25 indicated the presence of a GSH metabolite of 1,2-dichloroethane that does not contain a nucleoside. A similar hydrophilic peak was obtained for the hydrolysis products of polynucleotides from a glutathione plus cytosol incubations in which the polynucleotides instead of being added prior to the incubation were added after the incubation. The products of the glutathione plus cytosol metabolism of [^{14}C]-1,2-dichloroethane appear to be glutathione metabolites that were co-isolated with the polynucleotides rather than covalently bound adducts. In conclusion, covalently bound adducts were identified for microsome mediated binding of 1,2-dichloroethane to polynucleotides, while no evidence was obtained for glutathione plus cytosol mediated covalent binding to polynucleotides.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 9
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77)

REPRODUCED BY NATIONAL TECHNICAL INFORMATION SERVICE U.S. DEPARTMENT OF COMMERCE SPRINGFIELD, VA. 22161

EPA/600/J-85/139
JOURNAL ARTICLE

TOXICOLOGY AND APPLIED PHARMACOLOGY 78, 428–435 (1985)

Glutathione plus Cytosol- and Microsome-Mediated Binding of 1,2-Dichloroethane to Polynucleotides

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Received July 14, 1984; accepted December 21, 1984

Glutathione plus Cytosol- and Microsome-Mediated Binding of 1,2-Dichloroethane to Polynucleotides. LIN, E. L. C., MATTOX, J. K., AND PEREIRA, M. A. (1985). *Toxicol. Appl. Pharmacol.* 78, 428–435. 1,2-[1,2-^{14}C]Dichloroethane was metabolized by rat hepatic microsomes to products that irreversibly bound polynucleotides. The polynucleotides were then enzymatically hydrolyzed and the products separated by a high-performance liquid chromatograph (HPLC) equipped with an ODS or a SCX column. The products of microsome-mediated binding were identified in the HPLC eluate as 1,N^6-ethenoadenosine to polyadenylic acid, 3,N^4-ethenocytidine to polycytidylic acid, and two cyclic derivatives to polyguanylic acid. 1,2-[1,2-^{14}C]Dichloroethane was also metabolized in the presence of a glutathione (GSH)–cytosolic fraction and a polynucleotide. After enzymatic hydrolysis of the polynucleotide, the major peak of radioactivity was eluted from a Sephadex G-25 column in the salt volume which would exclude the presence of a product containing both GSH and a nucleoside. Chromatography by ODS–HPLC of the major peak from Sephadex G-25 indicated the presence of a GSH metabolite of 1,2-dichloroethane that did not contain a nucleoside. A similar hydrophilic peak was obtained for the hydrolysis products of polynucleotides from a glutathione plus cytosol incubation in which the polynucleotide instead of being added prior to the incubation was added after the incubation. The products of the glutathione plus cytosol metabolism of 1,2-[1,2-^{14}C]dichloroethane appear to be glutathione metabolites that coisolated with the polynucleotides rather than covalently bound adducts. In conclusion, covalently bound adducts were identified for microsome-mediated binding of 1,2-dichloroethane to polynucleotides, while no evidence was obtained for glutathione plus cytosol-mediated covalent binding to polynucleotides. © 1985 Academic Press, Inc.

1,2-Dichloroethane (DCE) is a large-volume synthetic organic chemical, with application in industry, agriculture, and commerce. Chronic administration of DCE by gavage induced a variety of tumors in rats and mice (National Cancer Institute, 1978b), but it did not produce tumors in lifetime inhalation studies (Maltoni *et al.*, 1980). DCE possessed weak direct mutagenic activity in the Ames *Salmonella* assay that was not increased by the addition of a standard hepatic S-9 fraction (McCann *et al.*, 1975; Rannug *et al.*, 1978). However, Rannug *et al.* (1978), Guengerich *et al.* (1980), Van Bladeren *et al.* (1981), and Reitz *et al.* (1982) found that a cytosol fraction supplemented with glutathione (GSH) greatly increased the mutagenicity of 1,2-DCE in *Salmonella*. Both GSH plus cytosol-mediated (Guengerich *et al.*, 1980; Ozawa and Guengerich, 1983) and microsome-mediated (Banerjee and Van Duuren, 1979; Banerjee *et al.*, 1980; Guengerich *et al.*, 1980) *in vitro* binding of 1,2-DCE to DNA and polynucleotides have been reported. In this communication, we report the identity of the products formed by microsome-mediated binding of DCE to polyadenylic acid (poly(A)), polycytidylic acid (poly(C)), and polyguanylic acid (poly(G)) and the lack of GSH plus cytosol-mediated

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covalent binding of DCE to the same polynucleotides.

METHODS

Animals and chemicals. Sprague–Dawley rats were purchased from Charles River (Portage, Mich.) and received Purina Rodent Chow (Ralston Purina Co., St. Louis. Mo.) and distilled water *ad libitum*. 1,2-[1,2-^{14}C]Dichloroethane and [*glycine*-^{3}H]glutathione were purchased from New England Nuclear Corporation (Boston, Mass.); 1,2-dichloroethane was from Fluka Chemical Corporation (Hauppauge, N.Y.); poly(A), poly(C), poly(G), 3,N^4-ethenocytidine (EtoCyd), 1,N^6-ethenoadenosine (EtoAdo), nuclease P_1, alkaline phosphatase, and acid phosphatase were from Sigma Chemical Company (St. Louis, Mo.). Both EtoAdo and EtoCyd were at least 97% pure by high performance liquid chromatography (HPLC). 7-(2-Hydroxyethyl)guanosine (HOET-Guo) was synthesized as described by Brookes and Lawley (1961).

Preparation of microsomal and cytosolic fractions. All animals were fasted overnight and killed by decapitation. All steps in the subcellular fractionation were performed at 0 to 4°C. The liver was homogenized in four parts (w:v) of 0.05 M potassium phosphate buffer, pH 7.4, containing 1.15% potassium chloride. The homogenate was centrifuged sequentially at 12,000*g* for 30 min and at 105,000*g* for 75 min. The resulting 105,000*g* supernatant fraction and pellet (resuspended in the same buffer) were again centrifuged at 105,000*g* for 60 min. The supernatant fraction was used for the cytosolic incubation, and the microsomal pellet was resuspended in 0.1 M potassium phosphate, pH 7.4, to give a suspension containing 22 to 25 mg of protein/ml. The protein concentration was measured by the procedure of Lowry *et al.* (1951).

Cytosolic and microsomal incubation. The incubation mixture contained 6 mg polynucleotide, 0.5 mM [^{14}C]DCE (24 μCi), 0.1 M potassium phosphate, pH 7.4, and 10 to 12.5 mg protein of either the microsomal or the cytosolic fraction in a final volume of 5 ml. In addition, the cytosolic incubation also contained 5 mM glutathione and at times [^{3}H]glutathione (21.5 μCi), while the microsomal incubations contained a NADPH-regenerating system consisting of 8 mM glucose-6-phosphate, 1 U/ml glucose-6-phosphate dehydrogenase, and 0.5 mM NADP. The incubations were performed under air in a sealed scintillation vial at 37°C for 3 hr. The incubations were stopped by placing them into an ice bath, followed by the addition of sodium dodecyl sulfate (SDS; final concentration 1%). The polynucleotides were then extracted with Kirby's phenol reagent (Kirby, 1967). In the cases where the polynucleotides were added after the incubation, they were added immediately before the phenol extraction. The polynucleotides were precipitated by the addition of 3 vol of alcohol containing 0.1 M sodium acetate and were washed four times by dissolving them in 0.1 M sodium acetate, pH 7.0, followed by precipitation with alcohol. The polynucleotides from cytosol incubation were further purified by Sephadex G-25 (Pharmacia, Piscataway, N.J.) column (0.9 × 86 cm) chromatography and eluted with 0.05 M ammonium formate, pH 6.8, to remove additional low-molecular-weight contaminants. The polynucleotides then were dissolved in water and quantified by absorbance at 260 nm.

Enzymatic hydrolysis of polynucleotides. The polynucleotides were dissolved in 0.1 M sodium phosphate, pH 6.5, containing 0.005 M magnesium chloride and hydrolyzed by a mixture of nuclease P_1 (0.1 mg/ml), acid phosphatase (2 mg/ml), and alkaline phosphatase (0.3 mg/ml) at 45°C for 18 hr. The solution was heated in a boiling water bath for 5 min and centrifuged to remove the denatured protein.

HPLC analysis of adducts. The polynucleotide degradation products were separated on a SP 8000 HPLC (Spectra Physics, Santa Clara, Calif.) equipped with a Magnum 9 Partisil 10 ODS-3 or SCX column (Whatman Inc., Boston, Mass.). The mobile phases used for the ODS column were 5% methanol for 10 min, a linear gradient to 40% methanol for 20 min, and then to 100% methanol for 10 min. The SCX column was eluted with 0.01 M ammonium formate, pH 4.0, for 10 min, followed by a linear gradient to 0.18 M ammonium formate, pH 4.0, in 10% methanol for 20 min, and continued with the last buffer for 20 min.

RESULTS

Isolation and Identification of the Microsome-Mediated Adducts

The products of microsome-mediated binding of 1,2-[1,2-^{14}C]DCE to polynucleotides after enzymatic hydrolysis were separated by a HPLC equipped with either an ODS (Fig. 1) or SCX (Fig. 2) column. The major radioactive peak in the elution profiles by both ODS and SCX chromatography for poly(A) and poly(C) coeluted with EtoAdo and EtoCyd, respectively. The elution profile for poly(G) contained two radioactive peaks from ODS fractions (fractions 48 and 52) and one radioactive peak from SCX fractions (fraction 64) in the region where Ado, EtoAdo, and EtoCyd were eluted. Oesch and Doerjer (1982) showed that 3,N^4-ethenoguanine was eluted from an ODS column between adenine and 1,N^6-ethenoadenine.

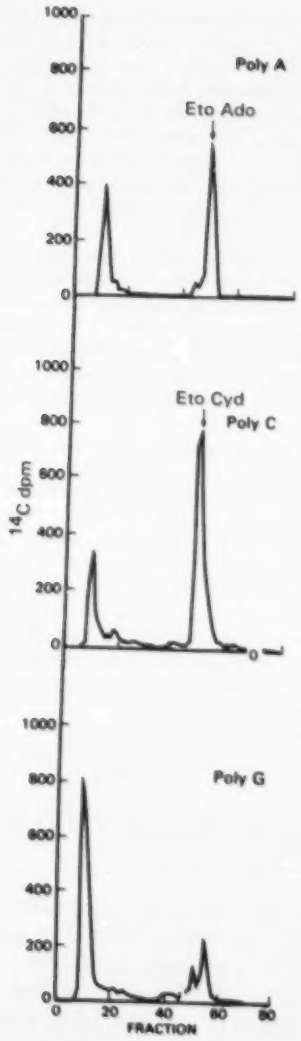

FIG. 1. Elution profile from ODS column chromatography of hydrolysates of polynucleotides incubated with DCE and microsome. The polynucleotides were hydrolyzed enzymatically with nuclease P_1, acid phosphatase, and alkaline phosphatase and applied to a Whatman Partisil 10 ODS-3 magnum-9 column in a volume of 1.6 to 1.8 ml. The column was eluted with 5% methanol for 10 min, followed by a linear gradient to 40% methanol for 20 min, then to 100% methanol for 10 min. The flow rate was 4 ml/min, and 2-ml fractions were collected. $1,N^6$-Ethenoadenosine and $3,N^4$-ethenocytidine were added to poly(A) and poly(C) hydrolysates, respectively. Absorbance was monitored at 260 nm.

The etheno derivative of deoxycytidine also elutes between deoxyadenosine and ethenodeoxyadenosine (data not shown). It seems that the etheno group is the deciding factor in the eluting time of the various etheno derivatives. Therefore, etheno derivatives of guanosine, N^2,3- and $1,N^2$-ethenoguanosine, would also be expected to be eluted in this region. 7-Hydroxyethylguanosine was eluted much earlier in fractions 19 and 25 from ODS and SCX columns, respectively. The order of binding of 1,2-DCE to the polynucleotides was poly(C) > poly(A) ⋙ poly(G) (Table 1).

Products of GSH plus Cytosol Incubations of DCE and Polynucleotides

After 1,2-[1,2-^{14}C]DCE was incubated with polynucleotides in the presence of GSH plus cytosol, the polynucleotide was extracted with phenol and low-molecular-weight contaminants of the polynucleotide were removed by Sephadex G-25 column chromatography. The Sephadex G-25 chromatography removed over 90% of the radioactivity present in the phenol-extracted DNA. Table 2 shows the amounts of 1,2-DCE metabolites present with with the polynucleotide in the high-molecular-weight material that was eluted in the void volume of the Sephadex G-25 chromatography. When the polynucleotide, instead of being added prior to the incubation, was added after a 3-hr incubation of 1,2-[1,2-^{14}C]DCE and GSH plus cytosol, the amount of 1,2-DCE metabolites that coeluted from the Sephadex G-25 with the polynucleotides was still approximately 50% of the amount that coeluted when the polynucleotide was added at the beginning of the incubation.

The polynucleotides were subjected to enzymatic hydrolysis and reapplied to the Sephadex G-25 column (Fig. 3). The elution profile for poly(C) and poly(G) contained two peaks of radioactivity, one in the void volume and one in the salt volume, while for poly(A) a third later eluting peak was also present at times. The same elution profile

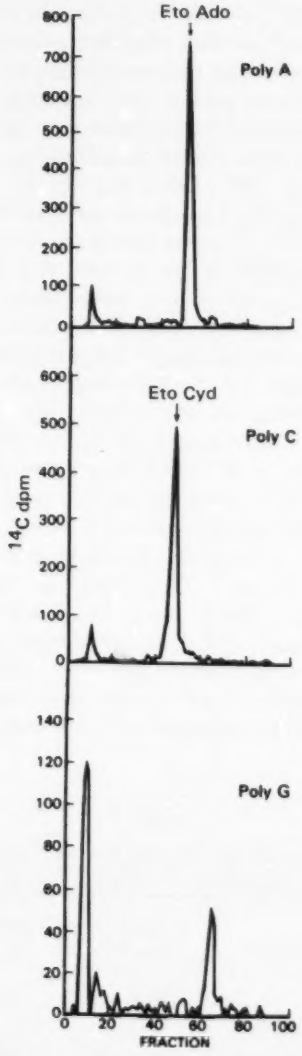

FIG. 2. Elution profiles from SCX column chromatography of microsome-mediated binding products of DCE to polynucleotides. The polynucleotides were degraded with nuclease P_1, acid phosphatase, and alkaline phosphatase and applied to a Whatman Partisil 10 SCX magnum-9 column in a volume of 1.6 to 1.8 ml. The column was eluted with 0.01 M ammonium formate, pH 4.0, for 10 min, followed by a linear gradient to 0.18 M ammonium formate, pH 4.0, in 10% methanol for 20 min. The flow rate was 4 ml/min, and 2-ml fractions were collected. 1,N^6-Ethenoadenosine and 3,N^4-ethenocytidine were added to poly(A) and poly(C) hydrolysates, respectively. Their absorbance was monitored at 260 nm.

was obtained for the control incubations (the polynucleotide added after the incubation) of the three polynucleotides as was obtained for the incubations of the respective polynucleotide. The disulfide, GSSG, and the major GSH plus cytosol metabolite of 1,2-DCE, $GSCH_2CH_2SG$, were eluted in fractions 20 and 19, respectively, and [^{3}H]GSH and 1,2-[1,2-^{14}C]DCE in the salt volume (fraction 26) similar to the major product of enzymatic hydrolysis. Therefore, the molecular weight of the material in the second and major peak eluted from Sephadex G-25 was substantially smaller than GSSG (MW 607) so that it cannot contain both GSH and a nucleoside but can contain GSH. The first peak eluted from the Sephadex G-25 column was in the void volume and probably contained either unhydrolyzed polynucleotide and/or contaminating protein.

The second and major radioactive peak from the enzyme-hydrolyzed polynucleotide that was eluted from Sephadex G-25 was subjected to HPLC–ODS chromatography (Fig. 4). Radioactivity from all the polynucleotides including the control incubations in which the polynucleotides were added after the incubation and just prior to the phenol extraction was eluted early in the hydrophilic region of the ODS column (fraction 9). Methylated, ethylated, and hydroxyethylated nucleosides are eluted much later from the column (data not shown). GSH is eluted from the ODS column in this early hydrophilic region. However, the molecular weight of the material obtained from the second peak of the Sephadex G-25 chromatography is too small to contain both GSH and a nucleoside. Therefore, it would appear that the material in the second (major) peak eluted from the Sephadex G-25 column contains a metabolite of 1,2-DCE and GSH that was coisolated with the polynucleotide and was not covalently bound. Also, when the early peak eluted from ODS chromatography was subjected to SCX column chromatography the same three major peaks were obtained for poly(A), poly(C), and poly(G) and for

TABLE 1

MICROSOME-MEDIATED BINDING OF 1,2-DICHLOROETHANE TO POLYNUCLEOTIDES

Polynucleotide[a]	pmol/μmol/mg protein	pmol/μmol/g liver
Poly(A)		
1,N^6-Etheno-adenosine	0.547 ± 0.014[b]	10.9 ± 0.51
Poly(C)		
3,N^4-Etheno-cytidine	1.38 ± 0.02	27.5 ± 0.81
Poly(G)		
Peak 1	0.0533 ± 0.0070	1.07 ± 0.25
Peak 2	0.135 ± 0.021	2.71 ± 0.42

[a] Results are from ODS column chromatography.
[b] Results are $\bar{x}$ ± SE from triplicate incubations.

their control (polynucleotide added after the incubation) incubations. Therefore, the radioactivity from the GSH plus cytosol incubation that was isolated along with the polynucleotide appears to be contaminating metabolites of 1,2-DCE and not metabolites covalently bound to the polynucleotide.

DISCUSSION

1,2-Dichloroethane (National Cancer Institute, 1978b) and 1,2-dibromoethane (DBE; National Cancer Institute, 1978a; Wong *et al.*, 1982) have been shown to induce various types of tumors in mice and rats. These vicinal dihalogenated ethanes are mutagenic in the Ames *Salmonella typhimurium* test (McCann *et al.*, 1975; Rannug *et al.*, 1978; Guengerich *et al.*, 1980; Van Bladeren *et al.* 1981) and bind to DNA (Banerjee and Van Duuren, 1979; Banerjee *et al.*, 1980; Guengerich *et al.*, 1980; Ozawa and Guengerich, 1983). Two pathways of metabolism have been proposed for their genotoxicity. One pathway of metabolism is oxidative metabolism by the microsomal mixed-function oxygenases to haloacetaldehyde (McCann *et al.*, 1975; Guengerich *et al.*, 1980). Microsomal metabolism of 1,2-DBE would produce bromoacetaldehyde and vinyl chloride, and 1,2-DCE would produce chloroacetaldehyde. Vinyl chloride and chloroacetaldehyde bound DNA *in vitro* and *in vivo*, resulting in the formation of 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine (Green and Hathway, 1978; Laib *et al.*, 1981; Oesch and Doerjer, 1982). We found that microsome-mediated binding of 1,2-DCE to polynucleotides also results in the formation of the EtoAdo and EtoCyd moieties as well as two unidentified derivatives of guanosine, neither of which is 7-(2-hydroxyethyl)guanosine. The guanosine moieties might be N^2,3-ethenoguanosine and 1,N^2-ethenoguanosine which are formed when chloroacetaldehyde binds guanosine (Sattsangi *et al.*, 1977; Oesch and Doerjer, 1982). Microsomal metabolism does not increase the mutagenicity of 1,2-DCE in *Salmonella typhimurium* (McCann *et al.*, 1975, Guengerich *et al.*, 1980). However, chloroacetaldehyde which forms the same etheno derivatives in DNA as were formed by 1,2-DCE in polynucleotides was mutagenic (McCann *et al.*, 1975). Also short- and long-term *in vivo* exposure to vinyl chloride re-

TABLE 2

GSH PLUS CYTOSOL-MEDIATED ASSOCIATION OF 1,2-DICHLOROETHANE TO POLYNUCLEOTIDES

Polynucleotide[a]	pmol/μmol/mg protein	pmol/μmol/g liver
Poly(A)	5.18 ± 1.07[b]	532 ± 110
Control poly(A)[c]	1.52	156
Poly(C)	8.24 ± 0.35	846 ± 367
Control poly(C)	5.25	539
Poly(G)	15.2 ± 3.75	1561 ± 385
Control poly(G)	7.07	726

[a] Results are from the high-molecular-weight material in the void volume of the Sephadex G-25 prior to enzymatic hydrolysis.
[b] Results are $\bar{x}$ ± SE from triplicate incubations.
[c] All incubations were carried out in the presence of GSH plus cytosol. For the controls, the polynucleotides were added to the incubation mixture after the 3-hr incubation and just prior to the phenol extraction.

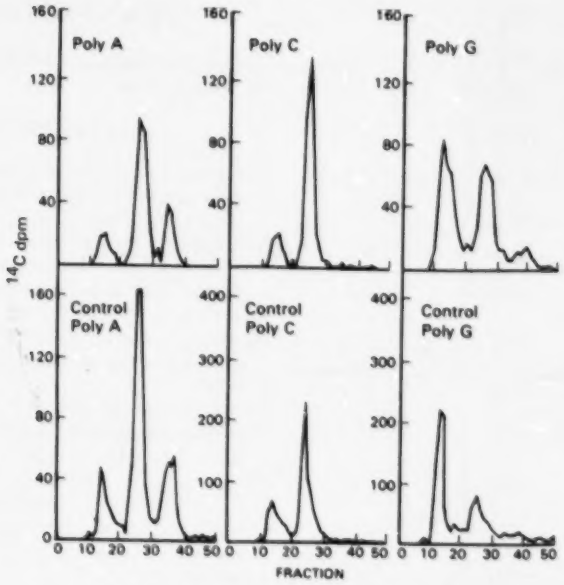

FIG. 3. Elution profiles of Sephadex G-25 column chromatography of enzymatically degraded polynucleotides that were incubated with GSH plus cytosol and DCE. The polynucleotides were degraded with nuclease P_1, acid phosphatase, and alkaline phosphatase, and 2- to 3-ml samples were applied to a 0.9 × 86-cm column and eluted with 0.05 M ammonium formate, pH 6.8. The flow rate was 0.2 ml/min, and 2-ml fractions were collected. The absorbance was monitored at 260 nm.

sulted in the formation of these etheno derivatives to DNA (Green and Hathway, 1978). Therefore, the formation of the etheno derivatives in DNA by microsomal metabolism of 1,2-DCE might be related to the carcinogenicity of 1,2-DCE.

The second pathway of metabolism of 1,2-DCE that has been proposed for its genotoxicity is cytosol-mediated conjugation to GSH followed by the formation of the mustard-type intermediate thiiranium (episulfonium) ion with the elimination of the second halogen (Guengerich *et al.*, 1980; Van Bladeren, *et al.*, 1981). This conjugation of 1,2-DCE to GSH is catalyzed by the GSH-*S*-transferase found in the cylosolic fraction of the liver. Though this conjugation pathway has been shown to result in the coisolation of ^{14}C from 1,2-DCE with DNA and polynucleotides (Guengerich *et al.*, 1980; Ozawa and Guengerich, 1983), covalent binding of 1,2-DCE as demonstrated by adduct formation has yet to be proven. While Ozawa and Guengerich (1983) have shown that the GSH plus cytosol-mediated binding of 1,2-DBE to DNA results in the formation of an *S*-[2-(N^7-guanyl)ethyl]glutathione adduct, they were unsuccessful in demonstrating the formation of this adduct as the result of the conjugation pathway metabolism of 1,2-DCE. We also were unable to demonstrate the formation of a covalent adduct of 1,2-DCE to polynucleotides as the result of metabolism by the conjugation pathway. Our results did demonstrate that GSH-conjugated metabolites of 1,2-DCE are tightly associated and coisolated with polynucleotides. Ozawa and Guengerich (1983) found a substantial amount of GSH-conjugated metabolites of 1,2-DBE which do not contain a nucleoside present in the DNA isolated from GSH plus cytosol incubation. Therefore, the use of the measurement of

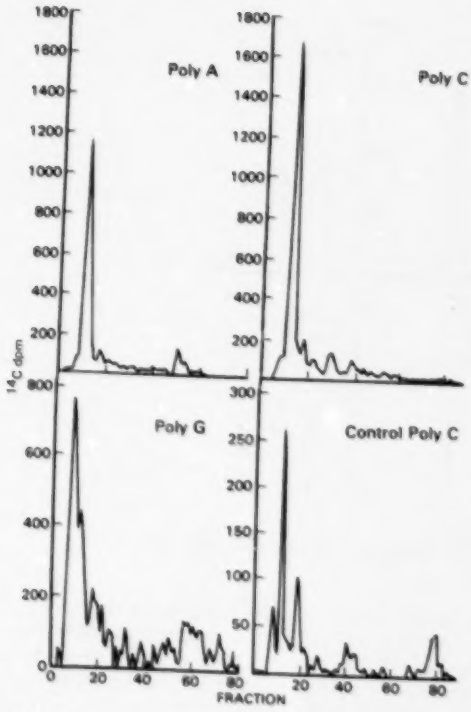

FIG. 4. Elution profiles of ODS column chromatography of enzymatically degraded polynucleotides that were incubated with GSH plus cytosol and DCE. Peak 2 from the Sephadex G-25 column chromatography of Fig. 3 was applied to a Whatman Partisil 10 ODS-3 magnum-9 column and eluted with 5% methanol for 10 min, followed by a linear gradient to 40% methanol for 20 min, then to 100% methanol for 10 min. The flow rate was 4 ml/min, and 2-ml fractions were collected.

radioactivity from halogenated ethanes that coisolate with polynucleotides and DNA rather than the measurement of the extent of adduct formation can result in an overestimation of covalent binding.

In conclusion, 1,2-DCE is metabolized by microsomes to a metabolite presumed to be chloroacetaldehyde that can bind with polynucleotides to form ethenoderivatives. Proof of the formation of adducts to nucleic acids by the GSH plus cytosol-mediated pathway is required before the relationship of this pathway to the carcinogenicity of 1,2-DCE can be evaluated.

ACKNOWLEDGMENT

The authors appreciate the excellent technical help of Mr. Gene A. Lease.

REFERENCES

BANERJEE, S., AND VAN DUUREN, B. (1979). Binding of carcinogenic halogenated hydrocarbons to cell macromolecules. *J. Natl. Cancer Inst.* **63,** 707–711.

BANERJEE, S., VAN DUUREN, B., AND ORUAMBO, F. (1980). Microsome-mediated covalent binding of 1,2-dichloroethane to lung microsomal protein and salmon sperm DNA. *Cancer Res.* **40,** 2170–2173.

BROOKES, P., AND LAWLEY, P. D. (1961). The alkylation of guanosine and guanylic acid. *J. Chem. Soc.* 3923–3928.

GREEN, T., AND HATHWAY, D. E. (1978). Interactions of vinyl chloride with rat-liver DNA in vivo. *Chem.–Biol. Interact.* **22,** 211–224.

GUENGERICH, P., CRAWFORD, W. M., DOMORADZKI, J., MACDONALD, T., AND WATANABE, P. (1980). *In vitro* activation of 1,2-dichloroethane by microsomal and cytosolic enzymes. *Toxicol. Appl. Pharmacol.* **53,** 303–317.

KIRBY, K. S. (1967). Isolation of nucleic acids with phenolic solvents. In *Methods in Enzymology* (L. Grossman and K. Moldare, eds.), Vol. 12, part B, pp. 87–99. Academic Press, New York.

LAIB, R., GWINNER, L., AND BOLT, H. (1981). DNA alkylation of vinyl chloride metabolites: Etheno derivatives or 7-alkylation of guanine? *Chem.–Biol. Interact.* **37,** 219–231.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., AND RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265–275.

MALTONI, C., VALGIMIGLI, L., AND SCARNATO, C. (1980). Long term carcinogenic bioassays of ethylene dichloride administered by inhalation to rats and mice. In *Banbury Report 5. Ethylene Dichloride: A Health Risk?* (B. Ames, P. Infante, and R. Reitz, eds.), pp. 3–29. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MCCANN, J., SIMMON, V., STREITWIESER, D., AND AMES, B. (1975). Mutagenicity of chloroacetaldehyde, a possible metabolic product of 1,2-dichloroethane (ethylene dichloride), chloroethanol (ethylenechlorohydrin), vinyl chloride, and cyclophosphamide. *Proc. Natl. Acad. Sci. USA* **72,** 3190–3193.

National Cancer Institute (1978a). *Bioassay of 1,2-Dibromoethane for Possible Carcinogenicity. NCI Carcinogenesis Technical Report Series, No. 86,* DHEW Publication No. (NIH) 78-1136. U.S. Govt. Printing Office, Washington, D.C.

National Cancer Institute (1978b). *Bioassay of 1,2-Dichloroethane for Possible Carcinogenicity. NCI Carcinogenesis Technical Report Series, No. 55,* DHEW

Publication No. (NIH) 78-1361. U.S. Govt. Printing Office, Washington, D.C.

OESCH, F., AND DOERJER, G. (1982). Detection of N^2,3-ethenoguanine in DNA after treatment with chloroacetaldehyde in vitro. *Carcinogenesis* **3,** 663–665.

OZAWA, N., AND GUENGERICH, F. P. (1983). Evidence for formation of an *S*-[2-(N^7-guanyl)ethyl]glutathione adduct in glutathione-mediated binding of the carcinogen 1,2-bibromoethane to DNA. *Proc. Natl. Acad. Sci. USA* **80,** 5266–5270.

RANNUG, U., SUNDVALL, A., AND RAMEL, C. (1978). The mutagenic effect of 1,2-dichloroethane on *Salmonella Typhimurium*. I. Activation through conjugation with glutathione in vitro. *Chem.-Biol. Interact.* **20,** 1–16.

REITZ, R., FOX, T., RAMSEY, J., QUAST, J., LANGVARDT, P., AND WATANABE, P. (1982). Pharmacokinetics and macromolecular interactions of ethylene dichloride in rats after inhalation or gavage. *Toxicol. Appl. Pharmacol.* **62,** 190–204.

SATTSANGI, P., LEONARD, N., AND FRIHART, C. (1977). 1,N^2-Ethenoguanine and N^2,3-ethenoguanine: Synthesis and comparison of the electronic spectral properties of these linear and angular triheterocycles related to the Y bases. *J. Org. Chem.* **42,** 3292–3296.

VAN BLADEREN, P., BREIMER, D., ROTTEVEEL-SMIJS, G., DE KNIJFF, P., MOHM, G., VAN MEETEREN-WALCHLI, B., BUIJS, W., AND VAN DER GEN, A. (1981). The relation between the structure of vicinal dihalogen compounds and their mutagenic activation via conjugation to glutathione. *Carcinogenesis* **2,** 499–505.

WONG, L., WINSTON, J., HONG, C., AND POLTNICK, H. (1982). Carcinogenicity and toxicity of 1,2-dibromoethane in the rat. *Toxicol. Appl. Pharmacol.* **63,** 155–165.

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